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A survey on the efficacy of tulathromycin in the treatment of infectious pneumonia of small ruminants in Iran

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Abstract

The principle aims of this study were to evaluate clinical, hematological, microbiological and macro and micro pathological effects of tulathromycin on small ruminants infectious pneumonia (IP) and to determine the side effects of the drug in the injected site. For this purpose, all ruminants (10 sheep and 9 goats) with signs of IP were assembled in the Khorasan-E-Razavi Province Veterinary Organization research centre. Also, all of these animals were free of internal parasite infestations and free of hydrated cysts in the lung tissue. Before tularthromycin injection, samples were taken as nasopharyngeal swabs for culturing *Pasteurella* spp. and *Mycoplasma* spp. and performing the Cell Blood Count (CBC) for blood. Each animal was injected at the dose of 2.5 mg/Kg subcutaneously. In 24-, 48- and 72-hours after injection, body temperature (BT), respiratory rate (RR), heart rate (HR), ruminal contraction rate (RCR), lung sounds (LS) and nasal discharges (ND) were recorded between 9-12 pm. Meanwhile on day 1 and day 3, the blood sampling was repeated and on day 3 nasopharyngeal swabs were repeated. Furthermore, 9 sheep and goats were killed on day 9 and the remainders were killed on the day 15 following injection and post-mortem inspections were conducted on lungs. From cases with lung lesions, a sample was taken for histopathological examination.

The data showed that:

A) The mean difference of BT, RR and HR of 0-, 24-, 48-, and 72-hrs after Tulathromycin injection was significantly different ($p< 0.05$).

B) The mean difference of No. WBC and % neutrophils of 0-, 24-, 48-, 72-hrs was significant ($p< 0.05$).

C) *Pasteurella* spp. was isolated from all sheep and goats before injection while this organism was cultured in only 2 of the animals on day 3 after tulathromycin injection ($p< 0.05$).

D) *Mycoplasma* spp. was cultured from 57.9% of the small ruminants and the results were identical in the second culturing with the exception of one animal. *Mycoplasma* spp. was cultured from 57.9% of the small ruminants and the results were identical in the second culturing with the exception of one animal.

E) 50% and 44.4% of the sheep and goats that were killed on the 9th and 15th days post-injection showed various pulmonary lesions including apical lob consolidation, apical lob consolidation plus adhesion, bronchopneumonia plus observed pleurisy.

On the basis of this investigation Tulathromycin has not had any antibacterial effect on *Mycoplasma* spp.

Keywords: Infectious pneumonia, Tulathromycin, Small ruminants

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Introduction

Respiratory infectious diseases are a major problem in all ruminant species and because of their contagious nature, they are most often observed as a 'herd problem' (Vogel, 1998). The infection rate is progressive and the incidence rate can reach 10-50% of the herd, depending on factors like age, immune-competence, infection pressure, and stress factors (Radostits, 2007). Infectious pneumonia (IP) and pleuropneumonia are diseases with complex aetiologies. Causative pathogens of these illnesses include different bacterial, mycoplasma and viral agents. The pathology is further complicated by environmental and management conditions. Due to their mortality, the cost of treatment, reduction of weight gain, milk yield and reproductive performance and finally decreasing of economic life span of the affected animals, the economic losses of the diseases are very high. Reduction of economic losses of IP is mostly a matter of management such as better housing, lowering the severity of stress conditions, appropriate vaccination schedule, immediate diagnosis and treatment in cases of disease (Blowy, 2004; Radostits, 2007).

Treating the bacterial agents of IP complex is paramount, hence the use of antibacterials in therapy has become a standard practice (Blowy, 2004; Smith, 2001). Therefore, it is generally accepted that early diagnosis followed by an effective antibiotic is crucial (Radostits, 2007); because:

- Most animals respond positively to antibiotics.
- Pulmonary lesions have a greater chance of complete repair.
- It prevents the invasion of opportunistic bacteria.

Choosing single or combined antibiotics should be based on (Hoar, 1 998; Roth, 2000; Schunith, 2002; Smith, 2001 and Vogel, 1998):

- Highest efficacy (lowest MIC for the target pathogens)
- Highest activity in the lung tissue (The drug should be concentrated in the infected lung.)
- Number of re-treatments (One shot is ideal.)
- Ease of use (low injection volume, use by unskilled persons)
- High safety (lowest toxicity)
- Short withdrawal time
- Lowest cost

Tulathromycin is a new molecule of Triamilide, a member of macrolides class ofantibiotics. The drug is effective in killing the gram negative spectrum of bacteria. In addition, the published studies have demonstrated its efficacy in treating IP in cattle and pigs (Pfizer Inc., 2004).

The question arose whether tulathromycin would be a good candidate for treating ovine and caprine IP. To address this question, we conducted a field study of the efficacy of tulathromycin on IP in sheep and goats.

Materials and methods

In autumn and winter of 2005, 10 sheep and 9 goats with clinical signs of IP including fever, nasal discharge, drowsiness and dysponea (Apley, 1999; Hoar, 1998; Perino and Apley, 1998) were used in the study. Also, all the animals were negative for G.I. parasites and lungworms on faecal examination (Booker, 1997; Hoar, 1998; Vogel, 1998) and they were free of hydrated cysts on X-ray of the thorax. The animals were kept in the veterinary research centre of the Khorasan-E-Razavi province veterinary organization. During the study, rectal temperature (RT), heart rate (HR), respiratory rate (RR), ruminal contraction rate (RC), nasal discharge score (ND) and quality of respiratory sounds (RS) were recorded on a daily basis.

Nasopharyngeal swabbing for culturing *Pasteurella* spp. and *Mycoplasma* spp. (Baron and Finegold, 1990) and blood samples for WBC count were collected prior to treatment

and three days after treatment.

The animals were treated with a single dose of tulathromycin at 2.5 mg/Kg administered subcutaneously, in the upper third of the thorax, posterior to the shoulder.

To evaluate injection site reactions, observation and touch of injection sites were recorded daily up to 15 days of post-treatment.

Drug efficacy was evaluated on the basis of the following indicators (15):

1-BT

2-ND score (0: normal, 1: seromucous, 2: mucopurulent)

3-LS score (0: normal, 1: crackling or wheezing)

4-RC score (0: normal, 1: weak, 2: atonia)

5-General appearance (0: normal, 1: dull, 2: recumbent.)

If any treated animal died or continued to show fever and dyspnoea, the treatment was considered as a failure. If any of the treated animals showed to pneumonic symptoms again in 9 days of post- initial treatment, such cases were labelled as 'relapse'.

In order to detect the final outcomes of the treatment, nine of the animals were killed on day 9 and the others were killed on day 15 of post-tulathromycin injection and post-mortem inspection was done for each animal. Any case with lung lesions were sampled for histopathological examination.

All data were statistically analysed using SPSS11 software. Quantitative parameters were evaluated by the method of paired-sample *t*-test and descriptive data were examined by Wilcoxon rank sum test.

Results

Tulathromycin ($p<0.05$) reduced fever within 24 hours of post treatment, and this situation continued through 3 days. The drug also reduced ($p<0.05$) Heart Rate and Respiratory Rate of the treated animals (see Table 1). The effects of the treatment on pneumonia severity indicators (PSI),WBC and neutrophil and lymphocyte counts were as expected, but the reduction of N/L ratio was not statistically significant (see Table2). Before treatment *Pasteurella* spp. were cultured from all nasopharyngeal swabs, while on day 3, only 2 nasopharyngeal swabs were *Pasteurella* positive. Therefore, tulathromycin removed ($p<0.05$) *Pasteurella* spp. from the lungs (see Table3). Before treatment, *Mycoplasma* spp. was cultured from 11 nasopharyngeal swabs. On day 3 post-treatment, 10 nasopharyngeal swabs of these animals were still *Mycoplasma* spp. positive. Hence, in this study, tulathromycin did not reduce *Mycoplasma* spp. from the lungs (see Table 3). In this study, there were no side effects at the injection sites (see Table 3). Nine animals were killed on the 9th day and the rest were killed on the 15th day of the post-treatment. At the post-mortem examination, 47.41% of the animals had lung lesions. On days 9 and 15, 50% and 44.4% of the animals were positive for pulmonary lesions, respectively.

In addition, the absence of *Mycoplasma* spp. in the first nasopharyngeal samples in 77.7% of the animals was associated with no lesions in the lung tissue (see Table 3). Thus, in this respect, the difference between the animals without lung lesions and those with lung lesions was statistically significant ($p<0.05$). In this study 50% of the sheep and 44.4% of the goats had lung lesions (Table 3) and the differences were not statistically significant.

In this survey, 47.4% of the animals had apical lobe hepatization macroscopically which was accompanied with adhesion in 44.4% (see Table 3). Histopathological examination of the solid lobes indicated that in 88.8% of the cases there were bronchopneumonia and only in 11.1% of the cases bronchopneumonia plus pleurisy were present (see Table 3).

Discussion

On the basis of the results of this survey it is clear that tulathromycin significantly

reduced the severity of clinical pneumonia symptoms (see Tables 1, 2 and 4). These findings agree with the results of field trials done with this drug in comparison with others including Ceftiofur curing lung infectious disease in calves (Bazargani, 2005. unpublished data). In addition, Pfizer performed a comparative study in evaluating the efficacy of tulathromycin opposed to Tilmicosin in 389 pneumonic calves of three weeks to 12 months old. In this trial, the cure-rate of tulathromycin was 99.2% versus 89.1 % for Tilmicosin (Pfizer Inc., 2004).

In another study, the potencies of tulathromycin and Florfenicol in the treatment of cattle infectious pneumonia were compared to each other. This survey was comprised of 108 animals and the two drugs cured 85% and 81% of the affected cattle in this trial, respectively. The efficacy difference was not statistically significant (Pfizer Inc., 2004).

Lack of precise indicators to determine the progressiveness of lung lesions complicates the precise judgment of the treatment efficacy. Therefore, it is appropriate to divide the animals in the experiment into three following groups (Apley, 1999) according to their clinical status:

1-Animals in a progressed stage of pneumonia

2-Animals in the early stage of the disease

3-Animals which are misdiagnosed

To evaluate the treatment effect of a drug in the first group of the animals the investigator would need to continue the observation for 24-48 hrs. Animals that die in this duration usually have progressed lesions in lungs at necropsy. None of the used sheep and goats in this study belong to this group. It is useful to mention that a suitable drug given to infectious pneumonic animals in early state of the disease must cure 75-85% of the affected animals (Radostits, 2007). In this trial no one of the animals died, so tulathromycin demonstrated an expected clinical effect.

Basic quantitative indicators like BT and RR, together with relevant qualitative symptoms such as RS, are a sound basis for diagnosis of IP (Perino and Apley, 1998; Radostits., 2007 and Reeve-Johnson, 1994). In the present study there was a statistically significant correlation coefficient between BT, RS, pneumonia severity indicators (PSI) and lung lesion in post killing examination (see Table 4).

Table 1. Clinical symptoms before and through 72 hrs after tulathromycin injection

Time (hr)	0	24	48	72
RT (°C)	40.68± 0.05	39.73± 0.06*	39.43± 0.08*	39.01± 0.09*
HR (No. per minute)	120± 2.7	113± 2.9*	109± 2.7*	93± 1.7*
RR (No. per minute)	33± 1.6	30± 1.2*	26± 1.0*	23± 094*

*Statistically significant difference (p<0.05)

Table 2. Pneumonia severity indicators and WBC counts before and 72hrs after injection

Time (hr)	0	24	48	72
PSI	4.84± 0.08	3.94± 0.12*	3.0± 0.17*	1.1± 0.2*
Total WBC/ml	11805± 604	-	-	9984+_375*
Total neutrophil/ml	6742± 433	-	-	4684± 288*
Total lymphocyte/ml	5084± 288	-	-	5289± 277
Rate of N/L[a]	1.37± 0.11	-	-	0.9± 0.06

* Statistically significant difference (p<0.05)
a N/L: Neutrophil/Lymphocyte

Table 3.Culture results, necropsy findings and condition of injected site before and after tulathromycin injection

No.	Species		Pasturella culture		Mycoplasma culture		Condition of injected site		Necropsy findings	
	sheep	goat	Before injection	After injection	Before injection	After injection	normal	abnormal	macro	micro
1	+		+	+	-	-	+		N	N
2	+		+	-	+	+	+		*	**
3	+		+	-	-	-	+		N	N
4		+	+	-	+	+	+		*	***
5		+	+	-	+	+	+		N	N
6		+	+	-	-	-	+		N	N
7	+		+	-	+	-	+		****	***
8	+		+	-	-	-	+		****	***
9	+		+	-	+	+	+		****	***
10	+		+	-	-	-	+		N	N
11	+		+	-	-	-	+		N	N
12		+	+	-	-	-	+		N	N
13		+	+	-	-	-	+		****	***
14	+		+	-	-	-	+		N	N
15	+		+	-	+	+	+		****	***
16		+	+	-	+	+	+		N	N
17		+	+	-	+	+	+		N	N
18		+	+	+	+	+	+		*	***
19		+	+	-	+	+	+		*	***

* Hepatization of apical lobe accompanied by adhesion
** Pleurisy and bronchopneumonia
*** Bronchopneumonia
**** Hepatization of apical lobe

Table 4. Correlation coefficient between lung lesions and clinical symptoms plus pneumonia severity indicators

Time (hrs)	0	24	48	72
HR (per minute)	-0.07	0.11	0.01	-0.01
BT (ºC)	0.05	0.60**	0.29	0.46*
RR (per minute)	0.53*	0.58**	0.47*	0.44
LS score	-	-	0.47*	0.71**
PSI	-0.01	0.32	0.36	0.54*

*statistically significant difference (p<0.05)
**statistically significant difference (p<0.01)

This finding agrees with that of Reeve-Johnson (Reeve-Johnson, 1994).

Although it has been claimed that WBC count is not a good indicator for diagnosis of IP in ruminants (14), the increased total number of neutrophils before treatment and its significant reduction after treatment (Table 2) clearly indicated that the animals under this investigation were suffering from bacterial IP. This observation agrees with the findings of other researchers (Kondraki, 2002 and Reeve-Johnson, 1994).

Small ruminant IP is considered either typical (pasteurelosis) or atypical (mycoplasmosis). However, in the majority of the field cases the two invaders are present, although with different frequency and pathogenicity (Pugh, 2002). In this survey *Pasteurella* spp. was cultured in 100% of the cases, while *Mycoplasma* spp. was isolated in 55.5% of the cases. Moreover, in either type of IP, consolidation of lung and pleurisy can be present, but in pasteurellosis pleurisy is usually fibrinous which can be diagnosed even macroscopically in post-mortem examination (Pugh, 2002). In the present study, post-

mortem examination of the animals revealed no cases of fibrinous pneumonia. The only case in which there was no *Mycoplasma* spp. growth was *Pasteurella* spp. positive in pre- and post-treatment had no pulmonary lesion in post-mortem examination (see Table 3). On the basis of the result of this study, the absence or the presence of *Mycoplasma* spp. in the beginning of the study has been associated in 77.7% animals with no lung lesion and in 70% of the cases with lung lesion, respectively (Table 3). And the degree of agreement between the two indicators (mycoplasma mycoplasma± and lung lesion±) is acceptable (kappa: 0.47 and p=0.03). It can be conceivable to say that *Mycoplasma* spp. was the most important agent for developing lung solidity. In 90% of the cases, tulathromycin had no effect on *Mycoplasma* spp... We conclude that tulathromycin is not an effective drug in the treatment of IP in small ruminants.

Considering that 50% of the animals had lung lesions on the 9th day, while on the 15th day pulmonary lesions were seen in 44.4% of the cases (Table 3), the differences between the two figures were not statistically significant. So, a reduction in frequency of lung solidity might be either an accidental event or a lung lesion resolution was taking place slowly (Pugh, 2002).

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بررسی اثرات درمانی تولترومایسین بر درمان پونومونی نشخوارکنندگان کوچک

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چکیده

هدف از انجام این مطالعه ارزیابی بالینی, خون شناسی، میکروب شناسی و اثرات آسیب شناسی میکرو و ماکرو داروی تولترومایسین بر پنومونی عفونی نشخوارکنندگان کوچک و همچنین عوارض سوء ناشی از دارو در محل تزریق است. به همین منظور 10 گوسفند و 9 بز با نشانه های پنومونی در مرکز تحقیقات اداره دامپزشکی خراسان رضوی جمع آوری شدند که تمامی آنها عاری از انگل های داخلی و کیست هیداتید ریوی بودند. سواپ بینی حلقی برای کشت پاستورلا و مایکوپلاسما و نمونه خون برای آزمایشات خون شناسی گرفته شد.تولترومایسین با دوز mg/kg2,5 به صورت زیرجلدی تزریق شد و در ساعتهای صفر،۲۴،۴۸ و 72 پس از تزریق درجه حرارت،تعداد تنفس ، ضربان قلب و تعداد حرکات شکمبه، صدای ریه و نوع ترشحات بینی در بین ساعات 9 تا 12 صبح ثبت گردید. در روزهای 1 و 3 نمونه گیری خون و روز 3 سواپ بینی تکرار شد.لازم به ذکر است که 2 گوسفند و بز در روز 9 و بقیه در روز 15 پس از تزریق ذبح شدند بازرسی پس از کشتار بر روی بافت ریه صورت گرفت و از موارد با جراحت ریوی یک نمونه بافتی برای آسیب شناسی گرفته شد.

نتایج حاکی از این بود که: 1) اختلاف معنی داری بین درجه حرارت بدن، ضربان قلبو تعداد تنفس در زمانهای صفر،۲۴،۴۸و 72 پس از تزریق تولترومایسین وجود دارد.2)اختلاف آماری معنی داری بین تعداد گویچه های سفید و تعداد نوتروفیل ها در زمانهای صفر،۲۴،۴۸و 72 پس از تزریق تولترومایسین وجود دارد.وجود دارد.3) در تمامی نمونه ها ی بینی پاستورلا جدا شد اما پس از تزریق تولترومایسین تنها در 2 مورد این باکتری از سواپ کشت شد.4) در 57,9% موارد قبل و بعد از تزریق تولترومایسین مایکوپلاسما کشت شد.5)جراحات ریوی مشاهده شده در نیمی از گوسفندان و 44% بزها شامل تراکم لوب آپیکال، جسبندگی برونکوپنومونی و پلورزی بود.

نتایج این طرح نشان می دهد که داروی تولترومایسین هیچگونه اثر ضد میکروبی بر روی مایکوپلاسما ندارد.

واژگان کلیدی:پنومونی عفونی،تولترومایسین ، نشخوارکنندگان کوچک